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Addition of 14 anchored loci to the porcine chromosome 8 comparative map

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Source/description: Porcine chromosome 8 is homologous to human chromosome 4, but a significant number of rearrangements in gene order exist when compared with human.[1] Genes expected to linkage map specifically in quantitative trait loci (QTL) regions for uterine capacity and ovulation rate[2,3] were chosen by their position on human chromosome 4 (UCSC version hg16 based on NCBI Build 34; Golden Path web site; http://genome.ucsc.edu/) and availability of EST sequences in TIGR Gene Indices (TIGR web site; http://www.tigr.org/tdb/ssgi/) that aligned to human genes. Primer pairs (Table 1) were designed using primer 3 (code available at: http://www-genome.wi.mit.edu/genome_software/other/primer3.html) to flank introns or 3′-UTR regions of porcine genes for amplification and SNP discovery.

PCR conditions/radiation hybrid panel: Polymerase chain reaction (PCR) was performed in a PTC-225 DNA engine (MJ Research Inc., Watertown, MA, USA) using 0.25 U Hot Star®

Table 1 Markers and genes mapped on SSC8.

Gene symbol: gene name	GenBank accession	Forward primer sequence Reverse primer sequence	Polymorphism[1]	Map[2]	Size (bp)	Nearest marker	Two-point LOD	Position (cM)[3]
HTRA3: serine protease HTRA3	BV102581	aagtctccccttgcaacct acactcctggacgtagctcct	_	R	450	*SW2410*	17.70	8:2
PGR1: T-cell activation protein	AY596450	atccagatcaagacgcaggt tgtcagccaatctccatcag	ttgggaggagRgagtgaaaca aagctagaggRaaaaagtggg	L	700	_	_	8:7
SB84: CHORI-242: 10M6 (PGR1)	AY596449	agggttctctggagaaacagaa gccaactccttaaaataagcctct	Microsatellite	L	148	_	_	8:7
SB87: CHORI-242: 10M6 (PGR1)	AY596448	ggctacagttctgctttgatcc catgtgtaggtaccaccaaaaa	Microsatellite	L	144	_	_	8:7
STK32: serine/threonine protein kinase	BV102996	ccacatacacctggaagacttc tgttgaatatgatgaattccttcc	gtgattgggcYgggctgctgc cgggggctggYagcttccccc	L	1300	_	_	8:14
MGC13159: hypothetical protein	AY596452	tcatttgcctgttgctgaac cccaaaaatgcacacagtga	gcctttaaccRagcctcagca	R, L	400	*S0098*	12.41	8:16
TEC: TEC-kinase	AY552751	cccgattccaaagcacaag atgccccagcccagatttc	gttttacttaYaatgtagaaa gaagcctttgMttctagacca	L	395	_	_	8:58
CXCL10: chemokine ligand 10	AY577902	cttggaggggtggcagtg attcagacatcttttctccccattc	aaagttaaacYtaaatgtctg	L	364	_	_	8:64
SCARB2: scavenger receptor class B, type 2	AY563473	gcattaacgagggatgcagag cgaacgagcacccctcatac	_	R	534	*S0086*	9.32	8:65
EDNRA: endothelin receptor A	AY540998	gtcgagaggtggcaaaaacag accaatatagtccatgaggagcag	ccaygtgctgYggtatatctg	L	589	_	_	8:72
IL15: interleukin 15	AY552750	atgccccagcccagatttc tggtacaaggagcctacaagagg	_	R	533	*S0069*	6.60	8:74
MAD2L1: mitotic arrest-deficient 2, homologue	TC118026	gagcaaggcattaccctacg tcgagtaaaggtttcagatgga	_	R	1000	*SW748*	11.36	8:77
CLGN: calmegin	AY536213	gacagcagggctcccaatag caagggctgctttctcttcc	aatagcttgtRttaaaccacc	L	359	_	_	8:78
RAP1: GTP-GDP dissociation stimulator 1	BV103513	atgaacctggaaaagaggcg acacattacccttttcctccc	_	R	350	*SW1671*	5.89	8:97
SCDR9: short chain dehydrogenase/ reductase 9	TC110509	acgtgccttggcagctataaat gtaacggcactgaaaaatgtga	_	R	181	*SSP1*	9.02	8:111
GPIG4: RasGEF domain family, member 1B	BV103553	taaacaacacacgttccagc aagccacagtccatacagag	_	R	250	*SW1980*	3.17	8:123

[1]Polymorphism targeted for genotyping is identified by IUB code.

[2]Markers placed on the IMpRH cR_{7000} radiation hybrid panel are designated with an R and those assigned on the Meat Animal Research Center (MARC) linkage map are shown by an L.

[3]Marker positions mapped on the radiation hybrid panel are interpolated from microsatellite positions on both maps.

Taq polymerase (Qiagen, Valencia, CA, USA), 1X of supplied buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.8 μM each primer, and 100 ng of genomic DNA in 25 μl reactions. The PCR mixture was held at 94 °C for 15 min, and cycled 40 times at 94 °C for 20 s, annealing for 30 s and extension at 72 °C for 1–1.5 min, followed by a final extension at 72 °C for 5 min. Amplicons containing SNPs were mapped using primer extension assay on the Sequenom MassArray™ system (San Diego, CA, USA). Uninformative amplicons were mapped using the IMpRH (cR_{7000}) 118-clone radiation hybrid (RH) panel[4] using primers described above, or designed from intron sequences obtained with the original EST primers (Table 1). Amplifications were performed as above except in 15 μl PCR using 12.5 ng panel DNA and 1 μM each primer.

Chromosomal location/linkage mapping: Linkage analyses were performed for SNPs across seven families (86 progeny) of the Meat Animal Research Center (MARC) swine reference population.[5,6] Multipoint locations for all mapped markers are based on the latest published swine genetic map[5] (http://www.marc.usda.gov/). Radiation hybrid data were analysed for two-point and multi-point linkage with the IMpRH mapping tool and submitted to the IMpRH database (http://imprh.toulouse.inra.fr/). Carthagene (http://www.inra.fr/bia/T/CarthaGene/) was used to estimate multi-point marker distance and order using all public markers on porcine chromosome 8 in the IMpRH database (http://imprh.toulouse.inra.fr/) and those developed in this study. Microsatellite markers were used to approximate position of RH mapped markers on the MARC linkage map (http://www.marc.usda.gov/). A total of 14 genes were assigned to either of the two maps (Fig. 1); five genes were mapped to SSC8p and nine genes to SSC8q (Table 1). One locus, *PGR1*, was mapped using two SNPs and two microsatellites (*SB84* and *SB87*) that were identified in a BAC containing the PGR1 gene. One small rearrangement was observed between 3 and 8.5 Mb on the human map, corresponding to about 2–16 cM on the porcine map. Genes (*QDPR* to *SCARB2*) ranging from 17 to 78 Mb on human chromosome 4 maintain the same order and orientation on porcine chromosome 8 from 26 to 65 cM. An inversion of gene order was defined between 80 and 150 Mb of human chromosome 4 (*GPIG4* to *EDNRA*) which corresponds from 70 cM to the end of the linkage group on pig chromosome 8, consistent with previous reports.[1] This breakpoint likely lies between *SCARB2* and *GPIG4* at 78 and 82 Mb, respectively.

Comments: Numerous QTL for several reproductive traits are located on porcine chromosome 8 (SSC8) and include ovulation rate (number of corpora lutea), uterine capacity or litter size,[2,7] age at puberty,[8] nipple number, and prenatal survival.[7] At least three QTL for ovulation rate in different regions of SSC8 have been identified in separate resource populations[2,9,10] and one QTL region for nipple number was confirmed in two populations.[7,8] Some of these QTL have been evaluated further[3,11] and associations of traits have been made with positional candidate loci.[12] The addition of these markers to the comparative map will help identify positional candidate genes in QTL regions.

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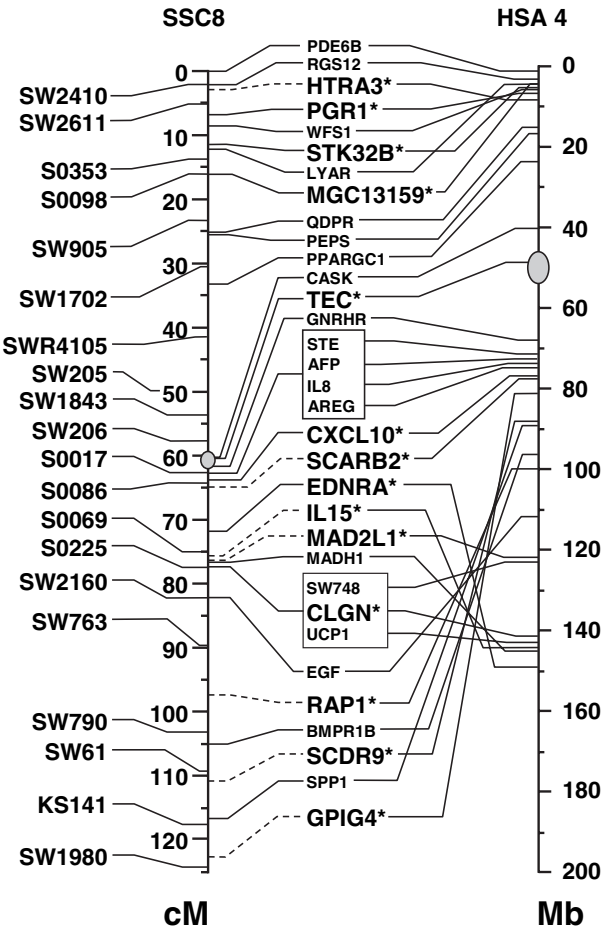

Figure 1 Comparative map of porcine chromosome 8 and human chromosome 4. Units are in centimorgans (cM; porcine) and megabases (Mb; human). New assignments are shown by an asterisk with selected public markers from the Meat Animal Research Center (MARC) genetic linkage map (http://www.marc.usda.gov/). Marker positions mapped on the IMpRH cR_{7000} panel are shown with a dotted line to the estimated position in the linkage map from microsatellite markers.

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Physical mapping of the *KCNJ8* gene to bovine chromosome 5q3.2–q3.4

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Source/description: The potassium inwardly rectifying channel, subfamily J, member 8 gene (*KCNJ8*), previously termed *KIR6.1*, maps to HSA 12p11.23 and encodes an integral membrane protein and inward-rectifier type potassium channel.[1] The human gene consists of three exons spanning about 9.7 kb and the encoded protein, which has a greater tendency to allow potassium to flow into a cell rather than out of a cell, is controlled by G-proteins.[1] *KCNJ8* is expressed preferentially in human heart muscle.[2] Studies of *KIR6.1* knock-out mice revealed a high rate of sudden death associated with spontaneous elevation on the electrocardiogram.[3] Administration of vasoconstrictive agents to these *KIR6.1* lacking mice leads to hypercontraction of the coronary arteries and thus a phenotype resembling variant angina in humans.[3] We screened the bovine RPCI-42 BAC library[4] with a radiolabelled bovine *KCNJ8* polymerase chain reaction (PCR) product to identify a *KCNJ8* clone in order to characterize the bovine gene. The PCR primers (F: 5′-GCG CTT GTC AAT CAC ATG G-3′, R: 5′-CCT CTG CTT TCC TCT TCT C-3′) were derived from a bovine *KCNJ8*-specific EST (GenBank accession number CB165999) which is homologous to characterized cattle *KCNJ8* sequences (accession numbers AF434905, AF434906). We identified one BAC clone (RP42-124E12) containing the *KCNJ8* gene. DNA was prepared from the BAC clone using the Qiagen plasmid midi kit (Qiagen, Hilden, Germany). The BAC insert size of 170 kb was determined by pulsed-field gel electrophoresis. The BAC DNA end sequences were obtained using the ThermoSequenase kit (Amersham Biosciences, Freiburg, Germany) and a LI-COR automated sequencer (LI-COR, Inc., Lincoln, NB, USA). The RP42-124E12 SP6 and T7 end sequences were deposited in the EMBL nucleotide database under accession numbers AJ781388, and AJ781389, respectively. A BLASTN sequence comparison of the bovine SP6 BAC end sequence with the build 34.3 of the human genome sequence revealed a significant match (BLAST E-value $6.4e^{-63}$) over 476 bp (identity = 73.1%) starting at 21 939 290 bp of HSA 12 approximately 130 kb downstream to human *KCNJ8*.

Primer sequences: Primers for PCR amplification of a 494 bp fragment were designed from the RP42-124E12 SP6 BAC end sequence (EMBL accession number AJ781388) using the PRIMER3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).
F: 5′-GAT TGT AAA TCC TCT GAA GAA TGT G-3′
R: 5′-CGC ATT CAT GAA AAC TGT GG-3′

Chromosomal location: The bovine BAC clone was labelled with digoxygenin by nick translation using a nick-translation mix (Boehringer Mannheim, Mannheim, Germany). Fluorescence *in situ* hybridization (FISH) on GTG-banded cattle chromosomes was performed using 750 ng of digoxigenin-labelled BAC DNA. About 1 μg sheared total bovine DNA and 10 μg salmon sperm DNA were used as competitors in this experiment. After hybridization over night, signal detection was performed using a Digoxygenin-FITC Detection Kit (Quantum Appligene, Heidelberg, Germany). The chromosomes were counterstained with 4′,6′-diaminidino-2-phenylindole (DAPI) and embedded in propidium iodide/antifade. Metaphase chromosomes that had been previously photographed by using a highly sensitive CCD camera were re-examined after hybridization with a Zeiss Axioplan 2 microscope (Zeiss, Jena, Germany) equipped for fluorescence. Identification of chromosomes followed strictly the international system for chromosome nomenclature of domestic bovids (ISCNDB 2000).[5] The bovine genomic BAC clone RP42-124E12 containing the *KCNJ8* gene was located to BTA 5q3.2–q3.4 by examination of metaphase chromosomes of 40 cells (Fig. 1).

Radiation hybrid mapping/PCR conditions: To confirm the cytogenetic assignment the 3000 rad Roslin/Cambridge bovine radiation hybrid (RH) panel[6] purchased from Research Genetics (Huntsville, AL, USA) was used to map *KCNJ8*. The PCR was carried out in a 20 μl reaction containing 25 ng of RH cell line DNA, 15 pmol of each primer and 0.75 U *Taq* polymerase (Qbiogene, Heidelberg, Germany). The reaction conditions started with a denaturing step at 94 °C for 4 min followed by

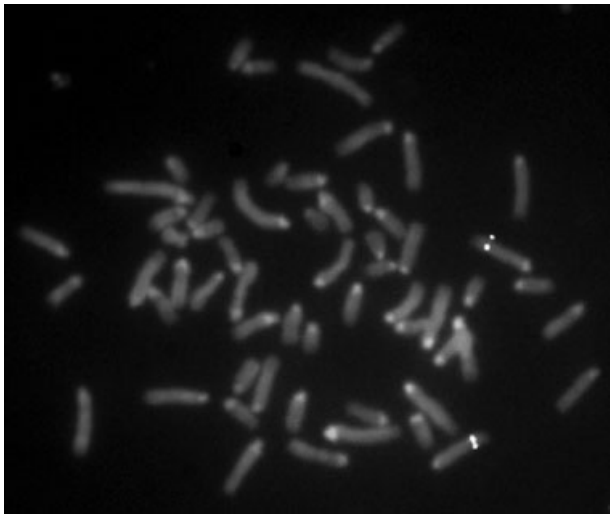

Figure 1 Chromosomal assignment of the bovine *KCNJ8* gene containing BAC RP42-124E12 by fluorescence *in situ* hybridization (FISH) analysis. Double signals are visible on both chromosomes BTA 5.